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Synthesis, antitubercular and antibacterial activity of some substituted quinazolinone analogs

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ABSTRACT

In recent years there is a tremendous increase of drug resistant pathogens, especially Mycobacterium tuberculosis leading to the design and development of newer antimycobacterial compounds. The reaction of various 3-amino-2-substituted phenyl quinazolin-4-one with chloroacetic acid provided 2-(4-oxo-2-substituted phenyl quinazolin-3(4H)-yl amino)acetic acid. The structure of the compounds has been confirmed by IR, [1]HNMR, Mass spectral data and Elemental analysis. Antitubercular and antibacterial activities were performed by microbroth dilution and cup-plate method respectively. The compounds have also been screened for antioxidant activity by DPPH method. Though the compounds showed moderate antioxidant activity, two compounds have shown good antitubercular activity and better antibacterial activity compared to the standard drug.

Key words: Quinazolin-4(3*H*)-one, antitubercular, antibacterial activity, antioxidant activity**.**

INTRODUCTION

A number of substituted quinazolin-3(4*H*)-ones were found to exhibit antitubercular [1], antibacterial [2], antimicrobial [3], anti-inflammatory [4] and CNS depressant [5] activities. In continuation of our research work on quinazolinone analogs herein we report the synthesis of title compounds 2-(4-oxo-2-substituted phenyl quinazolin-3(4*H*)-yl amino)acetic acid (2a-m)from 3-amino-2-substituted phenyl quinazolin-4-one and evaluation of their antitubercular, antibacterial and antioxidant activities. The intermediate 3-amino-2-susbtituted phenyl quinazolin-4-one (1) was obtained by the reaction of 2-susbtituted phenyl benzoxazin-4-one with hydrazine hydrate, the later compound was prepared by the reaction of substituted aryl acid chloride with anthranilic acid in pyridine. The 3-amino-2-susbtituted phenyl quinazolin-4-one (1) was reacted with chloroacetic acid in the presence of absolute alcohol to obtain the title compounds 2a-m (Figure 1, Scheme 1). Formation of the title compounds (2a-m) was confirmed by their IR, ^{1}HNMR, Mass and elemental analysis.

MATERIALS AND METHODS

Melting points were measured in open capillary tubes and are uncorrected. IR (KBR) spectra were recorded in film or in potassium bromide disks on a Perkin-Elmer 39 spectrophotometer (υ max in cm-1) and ^{1}H NMR spectra on a DPX 300 MHz Bruker FT-NMR spectrophotometer. The chemical shifts were reported as parts per million (δ ppm) using tetramethyl silane (TMS) as internal standard. Mass spectra were obtained on a JEOL-SX-102 instrument using fast atom bombardment (FAB positive). The progress of the reaction was monitored on a readymade silica gel

plates (Merck) using n-hexane: ethyl acetate as a solvent system. Spectral data (UV, IR, 1HNMR and Mass spectra) confirmed the structure of the synthesized compounds.

Synthesis of 3-amino-2-(substitutedphenyl) quinazolin-4(3*H*)-one (1):
2-(Substituted phenyl)-1,3-benzoxazin-4-one (0.01 mole) was taken in a round bottomed flask containing absolute alcohol, hydrazine hydrate (0.03 mole) and the contents were refluxed for 5 h. The reaction mixture was cooled to obtain the solid product and was recrystallized from alcohol.

Synthesis of 4-oxo-2-(substituted phenyl)-4*H*-quinazolin-3-ylamino)-acetic acid (2a-m).
3-amino-2-substituted phenyl quinazolinones (0.01m)were taken in a round bottomed flask and refluxed with absolute alcohol and chloroacetic acid (0.01m) for 4-5h. The reaction was monitored by TLC. After the completion of reaction contents were cooled to collect the solid crystals and recrystallized from absolute alcohol. All the compounds were prepared by following the similar procedure.

4-Oxo-2-phenyl-4*H*-quinazolin-3-ylamino)-acetic acid (2a):
$C_{16}H_{13}N_3O_3$, Mol wt: 295, m.p: 180-23, yield: 39.78%, λmax: 223, IR (KBR) cm-1: 3305 (Ar OH str), 3214 (Ar NH str), 3032 (cyc CH str), 1663 (Ar C=O str), 1561 (Ar C=C str), 1470 (Ali CH str), 765 (Ar CH out of plane bend). ^{1}HNMR (CDCl3) : δ(ppm) 8.29-8.31 (d,1H,NH), 7.47-7.81 (m,9H,ArH), 5.02 (s,2H,CH_2). m/e: 295.

2-(2-chloro-phenyl)-4-oxo-4*H*-quinazolin-3-ylamino)-acetic acid (2b):
$C_{16}H_{12}N_3O_3Cl$, Mol wt: 332, m.p: 160-62, yield: 81.96%, λmax: 221, IR (KBR) cm-1: 3285 (Ar OH str), 3083 (Ar NH str), 3012 (Ar CH str), 1674 (Cyc C=O str), 1612 (Ar C=C str), 1470 (Ali CH str), 1079 (Ar C-Cl str), 764 (Ar CH out-of-plane bend). ^{1}HNMR (CDCl3) : δ(ppm) 8.34-8.36 (d,1H,NH), 7.43-7.83 (m,7H,ArH), 4.97 (s,2H,CH_2). m/e: 334.

(4-oxo-2-*o*-tolyl-4*H*-quinazolin-3-ylamino)-acetic acid (2c):
$C_{17}H_{15}N_3O_3$, Mol wt: 309, m.p: 140-42, yield: 36.19%, λmax: 221, IR (KBR) cm-1: 3317 (Ar OH str), 3269 (Ar NH str), 3062 (Ar CH str), 1677 (Cyc C=O str), 1607 (Ar C=C str), 1470 (CH2, CH bend), 1468 (Ali CH str), 770 (Ar CH out-of-plane bend). ^{1}HNMR (CDCl3) : δ(ppm) 8.32-8.34 (d,1H,NH), 7.26-7.80 (m,7H,ArH), 4.86 (s,2H,CH_2), 2.28 (s,3H,CH_3). m/e: 310.

2-(4-chloro-phenyl)-4-oxo-4*H*-quinazolin-3-ylamino)-acetic acid (2d):
$C_{16}H_{12}N_3O_3Cl$, Mol wt: 332, m.p: 185-87, yield: 44.44%, λmax: 220, IR (KBR) cm-1: 3310 (Ar OH str), 3214 (Ar NH str), 2926 (Ar CH str), 1671 (Cyc C=O str), 1559 (Ar C=C str), 1470 (Ali CH str), 1091 (Ar C-Cl str), 769 (Ar CH out-of-plane bend). ^{1}HNMR (CDCl3) : δ(ppm) 8.23-8.25 (d,1H,NH), 7.44-7.87 (m,8H,ArH), 5.55 (s,2H,CH_2). m/e: 334.

2-(4-methoxy-phenyl)-4-oxo-4*H*-quinazolin-3-ylamino)-acetic acid (2e):
$C_{17}H_{15}N_3O_4$, Mol wt: 325, m.p: 183-84, yield: 32.91%, λmax: 221, IR (KBR) cm-1: 3304 (Ar OH str), 3217 (Ar NH str), 2967 (Ar CH str), 1673 (Cyc C=O str), 1607 (Ar C=C str), 1450 (Ali CH str), 1252 (Ar C-O-C str), 768 (Ar CH out-of-plane bend). ^{1}HNMR (CDCl3) : δ(ppm) 8.21-8.23 (d,1H,NH), 6.69-7.88 (m,8H,ArH), 5.54 (s,2H,CH_2), 3.31 (s,3H,CH_3). m/e: 326. Elemental analysis: Calcd%:Found% C 62.76:62.75, H 4.62:4.71, N 12.92:12.81.

2-(2-bromophenyl)-4-oxoquinazolin-3(4*H*)-ylamino)acetic acid (2f):
$C_{16}H_{12}N_3O_3Br$, Mol wt: 374, m.p: 135-38, yield: 54.05%, λmax: 221, IR (KBR) cm-1: 3312 (Ar OH str), 3262 (Ar NH str), 3058 (Ar CH str), 1677 (Cyc C=O str), 1601 (Ar C=C str), 1470 (Ali CH str), 1027 (Ar C-Br str), 768 (Ar CH out-of-plane bend). m/e: 376. Elemental analysis: Calcd%:Found% C 51.35:51.27, H 3.20:3.28, N 11.23:11.32.

2-(4-nitrophenyl)-4-oxoquinazolin-3(4*H*)-ylamino-acetic acid (2g):
$C_{16}H_{12}N_4O_5$, Mol wt: 340, m.p: 240-43, yield: 50.09%, λmax: 221, IR (KBR) cm-1: 3303 (Ar OH str), 3220 (Ar NH str), 3033 (Ar CH str), 1677 (Cyc C=O str), 1581 (Ar C=C str), 1519 (Ar NO2 str), 1470 (Ali CH str), 766 (Ar CH out-of-plane bend). m/e: 341. Elemental analysis: Calcd%:Found% C 56.47:56.42, H 3.53:3.61, N 16.47:16.37.

2-(4-oxo-2-*m*-tolylquinazolin-3(4*H*)-ylamino)acetic acid (2h):
$C_{17}H_{15}N_3O_3$, Mol wt: 309, m.p: 112-114, yield: 86.73%, λmax: 221, IR (KBR) cm-1: 3283 (Ar OH str), 3124 (Ar NH str), 2919 (Ar CH str), 1672 (Cyc C=O str), 1591 (Ar C=C str), 1433 (Ali CH str), 773 (Ar CH out-of-plane bend). m/e: 310.

2-(4-oxo-2-*p*-tolylquinazolin-3(4*H*)-ylamino)acetic acid(2i):
$C_{17}H_{15}N_3O_3$, Mol wt: 309, m.p: 150-53, yield: 60.24%, λmax: 221, IR (KBR) cm-1: 3308 (Ar OH str), 3200 (Ar NH str), 3033 (Ar CH str), 1686 (Cyc C=O str), 1609 (Ar C=C str), 1468 (Ali CH str), 774 (Ar CH out-of-plane bend). m/e: 310. Elemental analysis: Calcd%:Found% C 66.02:66.12, H 4.854.81, N 13.5913.66.

2-(3-chlorophenyl)-4-oxoquinazolin-3(4*H*)-ylamino)acetic acid (2j):
$C_{16}H_{12}N_3O_3Cl$, Mol wt: 332, m.p: 170-74, yield: 63.82%, λmax: 220, IR (KBR) cm-1: 3308 (Ar OH str), 3214 (Ar NH str), 2920 (Ar CH str), 1663 (Cyc C=O str), 1584 (Ar C=C str), 1469 (Ali CH str), 973 (Ar C-Cl str), 770 (Ar CH out-of-plane bend). m/e: 330. Elemental analysis: Calcd%:Found% C 58.27:58.36, H 3.64:3.58, N 12.75:12.61.

2-(2-nitrophenyl)-4-oxoquinazolin-3(4*H*)-ylamino)acetic acid (2k):
$C_{16}H_{12}N_4O_5$, Mol wt: 340, m.p: 189-90, yield: 82.98%, λmax: 234, IR (KBR) cm-1: 3309 (Ar OH str), 3214 (Ar NH str), 3033 (Ar CH str), 1672 (Cyc C=O str), 1600 (Ar C=C str), 1558 (Ar NO2 str), 1470 (Ali CH str), 780 (Ar CH out-of-plane bend). m/e: 341.

2-(3-nitrophenyl)-4-oxoquinazolin-3(4*H*)-ylamino)acetic acid (2l):
$C_{16}H_{12}N_4O_5$, Mol wt: 340, m.p: 219-20, yield: 89.33%, λmax: 230, IR (KBR) cm-1: 3306 (Ar OH str), 3220 (Ar NH str), 3033 (Ar CH str), 1676 (Cyc C=O str), 1639 (Ar C=C str), 1533 (Ar NO2 str), 1469 (Ali CH str), 780 (Ar CH out-of-plane bend). m/e: 341.

2-(3-methoxy phenyl)-4-oxoquinazolin-3(4*H*)-ylamino)acetic acid (2m):
$C_{17}H_{15}N_3O_4$, Mol wt: 325, m.p: 149-51, yield: 80.64%, λmax: 220, IR (KBR) cm-1: 3315 (Ar OH str), 3264 (Ar NH str), 3056 (Ar CH str), 1676 (Cyc C=O str), 1583 (Ar C=C str), 1430 (Ali CH str), 1287 (Ar C-O-C str), 767 (Ar CH out-of-plane bend). m/e: 326.

Biological Activity
Antitubercular Activity:
All the synthesized compounds were tested for their *invitro* antitubercular activity against *mycobacterium tuberculosis* by agar dilution method [6] with the use of Middlebrook 7H-9 broth and standard strain of *M. tuberculosis* $H_{37}Rv$. The basal medium was prepared according to manufacture's instructions (Hi-Media) and sterilized by autoclaving. 4.5 ml of broth was poured into each one of the sterile bottles. To this, 0.5ml of ADC supplement is added. This supplement contains catalase, dextrose and bovine serum albumin fraction. Then a stock solution of the compound was prepared (10mg / ml). From this appropriate amount of solution is transferred to media bottles to achieve final concentrations of 25, 50, 100ug / ml. Finally 10ul suspension of *M.tuberculosis* strain (100000 organisms/ml, adjusted by Mc Farland's turbidity standard) was transferred to each of the tube and incubated at 37ºC. Along with this one growth control without compound and drug controls were also maintained. The bottles were inspected for growth twice a week for a period of three weeks. The appearance of turbidity was considered as growth and indicates resistance to the compound. The growth was confirmed by making a smear from each bottle and performing a ZN stain. The results are produced in **Table 1.**

Antibacterial activity
All the synthesized compounds were tested for their antibacterial activity against both gram positive and gram negative organisms viz., *Bacillus subtilis* (NCIM 2697), *Staphylococcus auereus* (NCIM 2079), *Escherichia coli* (NCIM 2065) *and Klebsella pneumonia* (NCIM 5082). The activity was performed by following the procedure of cup plate agar diffusion method [7]. A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms. 0.1 mL of inoculums (of 10^4 to 10^6 CFU / mL population prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. Accurately measured (0.1 mL) solution of each sample and standard were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 ºC for 24 h. The presence of definite zones of inhibition around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of DMSO,

which was used as a solvent for sample. The diameter of the zone of inhibition was measured and recorded in **Table 1.**

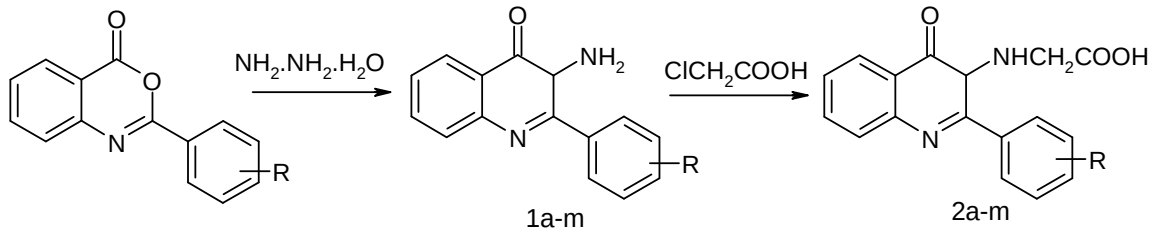
Antioxidant Activity

Free radical scavenging activity of the test compounds were determined by the 1,1- diphenyl picryl hydrazyl (DPPH) assay method [8]. Drug stock solution (1 mg mL–1) was diluted to final concentrations of 2, 4, 6, 8 and 10 mg mL–1 in methanol. DPPH methanol solution (1 mL, 0.3 mmol) was added to 2.5 mL of drug solutions of different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity. Methanol was used as the solvent and ascorbic acid as the standard. The standard drug used was ascorbic acid in a concentration of 10-40µg/ml. Results are presented in **Table 1**.

Table 1: Biological activity of the compounds 2a-m

		Antibacterial activity (Zone of inhibition in mm)				**Antitubercular activity (Conc in µg/ml)**	**Antioxidant activity (% inhibition)**			
Sl.No	Compd Code	*E.coli*	*K.pnemoniae*	*B.subtilis*	*S.aureus*	Concentration	50µg/ml	100 µg/ml	150 µg/ml	200 µg/ml
1	2a	20	19	15	15	>100	30.22	27.31	28.45	30.01
2	2b	15	20	16	17	>100	29.28	28.55	30.32	64.69
3	2c	20	19	15	17	>100	71.03	73.73	73.94	75.28
4	2d	18	18	15	17	>100	64.79	65.84	67.19	66.77
5	2e	20	15	17	18	>100	42.78	42.99	46.11	55.02
6	2f	18	16	15	16	>100	61.99	65.32	35.20	42.06
7	2g	15	19	16	15	>100	60.02	67.08	69.78	77.47
8	2h	19	15	15	12	>100	44.03	48.70	51.51	56.06
9	2i	20	18	16	17	>50	53.06	54.83	57.11	57.42
10	2j	19	16	14	15	>100	46.41	46.41	46.52	46.31
11	2k	20	17	13	15	>50	14.12	13.71	15.16	14.54
12	2l	20	19	16	13	>100	8.72	53.37	54.21	52.54
13	2m	19	16	16	18	>100	50.63	53.66	69.80	72.56
14	Streptomycin	25	26	20	20	--	--	--	--	--
15	Isoniazid	--	--	--	--	0.2	--	--	--	--
16	Rifampicin	--	--	--	--	0.2	--	--	--	--

Fig 1: Scheme 1



R

2-Cl 2-CH_3 2-NO_2 2-Br -H

3-Cl 3-CH_3 3-NO_2 3-OCH_3

4-Cl 4-CH_3 4-NO_2 4-OCH_3

RESULTS AND DISCUSSION

The antitubercular activity of the synthesized compounds revealed that the compounds 2-(2-nitro-phenyl)-4-oxoquinazolin-3(4*H*)-yl amino)-acetic acid (2k) and 2-(4-oxo-2-*p*-tolylquinazolin-3(4*H*)-yl amino)-acetic acid (2i) were active at a concentration of 50µg/ml against *Mycobacterium tuberculosis* while all other compounds were

effective at a concentration of 100µg/ml. All the synthesized compounds irrespective of various substitutions have shown moderate activity against both gram +ive and gram –ive bacterial strains. The antioxidant activity showed that the compounds (4-oxo-2-*o*-tolyl-4*H*-quinazolin-3-ylamino)-acetic acid (2c) and 2-(4-nitrophenyl)-4-oxoquinazolin-3(4*H)*-ylamino-acetic acid (2g) have profound antioxidant activity.

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